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# GROWTH OF FIVE PATHOGENIC FUNGI ON CELL WALL-RELATED MONOSACCHARIDES

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SUMMARY

Spores of *Monilinia fructicola*, *Colletotrichum coccodes*, *Botrytis cinerea*, *Alternaria alternata* and *Mucor mucedo* were transferred to agar plates containing 0.5, 5 or 50 mM concentrations of various plant cell wall-related monosaccharides or glucose. The wall-related monosaccharides tested were arabinose, galactose, galacturonic acid, mannose, rhamnose and xylose. Germ tube length was measured after 24 h. Growth of germ tubes on media containing the different monosaccharides varied substantially among fungi. The most striking response was with *M. mucedo*; spores were only able to grow on media containing glucose or galactose.

Increase in colony size was determined during a 7-day period after subcultures were transferred to media containing 5 mM arabinose, galactose, galacturonic acid, mannose, rhamnose and xylose, each with and without 5 mM glucose. Glucose, alone or in conjunction with any other monosaccharide, was the optimal carbon source for growth. In general, fungi were unable to grow as well on any of the wall-related monosaccharides without glucose. Galacturonic acid and rhamnose, major components of pectic polysaccharides of plant cell walls, were unable to support any growth of *M. fructicola* cultures. No wall-related monosaccharides supported more than 50% of the growth observed when *M. mucedo* cultures were grown on glucose.

*Key words:* Host-pathogen interaction — Carbon source — Galactose

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## INTRODUCTION

Although plants are constantly exposed to parasitic microorganisms, they generally remain resistant to severe invasion. However, many fruits naturally become more susceptible to fungal pathogens as they soften during ripening [1]. The nature of this decreased resistance of fruit tissues to fungal attack remains unclear.

Host plant cell walls represent an initial natural barrier and carbon source for fungal pathogens [2,3]. Thus, much research has been conducted on resistance-related alterations of cell walls such as callose deposition, lignification, suberization, and accumulation of calcium [2,4]. Recent evidence suggests that polysaccharides from higher plant and fungal cell walls are able to elicit phytoalexin accumulation in host plant cells [5,6]. However, little research has been conducted on specific aspects of host plant cell walls as a source of carbon for invading fungi [7—9].

Because fungal pathogens produce hydrolytic enzymes that degrade the carbohydrate network of plant cell walls, the individual wall polysaccharides and their monomeric constituents represent a source of carbon for fungal growth and energy production [8,9]. We are studying the role of cell wall structure and metabolism in relation to the increased susceptibility of fruit to fungal pathogens during ripening. The objective of this study was to determine the ability of five fungal pathogens to utilize cell wall-related monosaccharides for growth in culture.

## MATERIALS AND METHODS

Cultures of *A. alternata* (Fries) Keissler, *B. cinerea* (Persoon) Fries, *C. coccodes* (Wallroth) Huges, *M. fructicola* (Winter) Honey and *M. mucedo* (Linnaeus) Fresenius were maintained on potato dextrose agar. Continuous light was used to promote sporulation of *B. cinerea* and *C. coccodes* cultures. Spores were harvested from young, vigorously growing cultures, diluted with sterile distilled water to a concentration of $10^5$—$10^6$ spores/ml, and transferred to 15 × 100 mm Petri plates containing 10 ml of 1.5% Bacto Agar (Difco) amended with either 0.5, 5 or 50 mM arabinose, galactose, galacturonic acid, glucose, mannose, rhamnose or xylose. For each treatment, two Petri plates were inoculated with each of the five fungi. Petri plates containing only water agar served as controls (no carbon source). Spore germination was observed after 24 h and the length of germ tubes was measured at 100× with a Dietz compound microscope fitted with an ocular micrometer.

Growth of fungal colonies was determined by transferring spores and mycelium of each fungus with a sterile transfer loop to four Petri plates that were divided into four numbered quadrants. Inoculations were made at the edge of plates in each quadrant, allowing measurement of radial growth. Petri plates of each treatment contained 10 ml of water agar amended with

5 mM arabinose, galactose, galacturonic acid, mannose, rhamnose or xylose with and without 5 mM glucose. Measurements of fungal growth were recorded for 7 days.

The neutral sugar content of Bacto Agar was determined after incubating 10 mg of anhydrous agar in 2 N trifluoroacetic acid at 121°C for 1 h to hydrolyze any non-cellulosic polysaccharides present. Aldononitrile acetate derivatives of individual monosaccharides were estimated using gas chromatography as previously described [10]. No monosaccharides were detected in the agar at a sensitivity capable of detecting nanogram quantities.

All sugar solutions were filter sterilized before incorporation into agar media that had been sterilized by autoclaving at 121°C for 20 min.

## RESULTS AND DISCUSSION

Germ tube growth of *B. cinerea*, *A. alternata*, *M. fructicola*, *C. coccodes* and *M. mucedo* spores on agar containing no carbon source (water control) or one of three concentrations of glucose is presented in Table I. *B. cinerea*

TABLE I

GERM TUBE GROWTH OF FUNGAL SPORES ON WATER AGAR AND WATER AGAR AMENDED WITH GLUCOSE

Means are the average of 20 observations.

| Fungus | Glucose concentration (mM) | Germ tube growth (mm in 24 h)[a] | % of control |
|---|---|---|---|
| *B. cinerea* | 0 | 3.0 ± 1.3 | — |
| | 0.5 | 8.0 ± 2.5 | 270 |
| | 5 | 5.1 ± 1.7 | 174 |
| | 50 | 5.2 ± 2.2 | 176 |
| *A. alternata* | 0 | 4.4 ± 1.2 | — |
| | 0.5 | 4.7 ± 1.7 | 107 |
| | 5 | 3.5 ± 1.2 | 80 |
| | 50 | 3.6 ± 1.9 | 82 |
| *M. fructicola* | 0 | 2.4 ± 1.2 | — |
| | 0.5 | 5.8 ± 2.4 | 245 |
| | 5 | 6.2 ± 2.8 | 264 |
| | 50 | 4.9 ± 2.3 | 209 |
| *C. coccodes* | 0 | 4.6 ± 1.7 | — |
| | 0.5 | 5.1 ± 2.1 | 112 |
| | 5 | 4.4 ± 1.4 | 97 |
| | 50 | 7.6 ± 2.7 | 166 |
| *M. mucedo* | 0 | 0 | — |
| | 0.5 | 0 | — |
| | 5 | 5.9 ± 3.1 | — |
| | 50 | 3.8 ± 2.7 | — |

[a]Mean ± S.D.

and *M. fructicola* spores grew more rapidly on media containing glucose as a carbon source than on water agar. However, *A. alternata* and *C. coccodes* spores germinated and grew equally well on both media. *M. mucedo* spores did not germinate on water agar or on media containing 0.5 mM glucose.

Although glucose is a component of cellulose and hemicellulose polymers of plant cell walls, it is a readily utilizable substrate for growth and energy production and is a major soluble intermediate in most plant tissues. Therefore, in addition to the water agar control, glucose represents a suitable carbon source for comparing the growth of fungi on other wall-related monosaccharides. Substantial differences existed in the ability of germinating spores of the five pathogenic fungi to utilize the wall-related sugars (Fig. 1). Galactose was the only monosaccharide of all those tested, other than glucose, that supported germination and growth of *M. mucedo*; germ

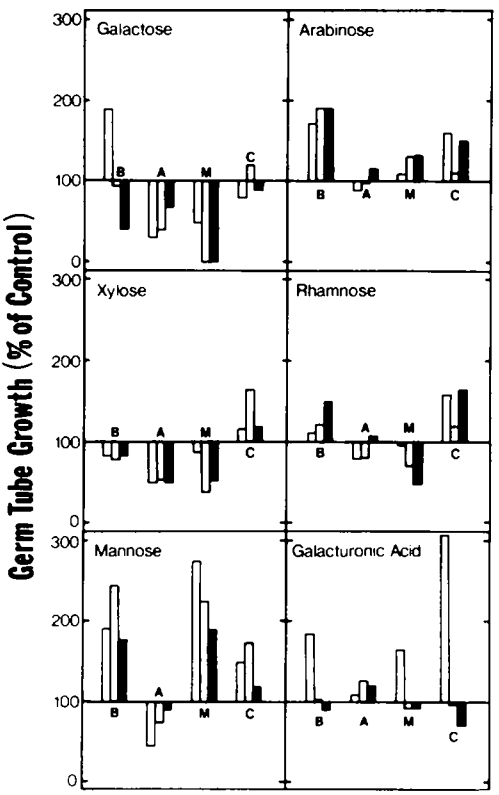


Fig. 1. Germ tube growth of fungal spores after 24 h on various cell wall-related monosaccharides. Growth is shown as percentage of water agar control values which are shown in Table I. Fungi are abbreviated as follows: *B. cinerea* (B), *A. alternata* (A), *M. fructicola* (M) and *C. coccodes* (C). The unshaded, lightly shaded and dark shaded bars represent 0.5, 5.0 and 50.0 mM concentrations, respectively, of the particular monosaccharides. Each value represents the mean of 20 germ tubes.

tubes grew 5.4 ± 2.8, 6.2 ± 3.6 and 4.8 ± 3.4 mm in 24 h on 0.5, 5 and 50 mM galactose, respectively. In contrast, germination of *M. fructicola* spores was completely inhibited by 5 and 50 mM galactose (Fig. 1). Galacturonic acid at a low concentration (0.5 mM) stimulated the growth of germinating *B. cinerea*, *M. fructicola* and *C. coccodes* spores. However, higher concentrations of this monosaccharide, which is the primary component of the pectic portion of plant cell walls, had little or no effect on germ tube growth. Another of the more striking differences was the growth of germinating spores on media amended with mannose. This sugar strongly stimulated the growth of *B. cinerea*, *M. fructicola* and *C. coccodes* germ tubes but had no effect on, or slightly inhibited, the growth of *A. alternata* (Fig. 1) and did not permit germination of *M. mucedo*.

The ability of cell wall-related monosaccharides to support the growth of established cultures of the five fungi was tested by transferring subcultures (mixture of spores and mycelia) of each fungus to agar amended with 5 mM galactose, arabinose, rhamnose, xylose, mannose or galacturonic acid, each with and without 5 mM glucose. Growth was measured by determining the diameter of each colony during incubation at 30°C for up to 7 days. Growth curves for all five fungi were sigmoidal when cultures were grown on agar amended with 5 mM glucose (Fig. 2) as well as with all other monosaccharides tested (data not shown). The growth of fungal colonies on the various monosaccharides after 4 days is shown in Fig. 3. As with spore growth, substantial differences existed in the ability of the fungi to utilize cell wall-related monosaccharides for growth. Glucose, alone or in combination with any other monosaccharide, supported the fastest rate of colony growth; fungal colonies were not able to grow as well on agar containing any of

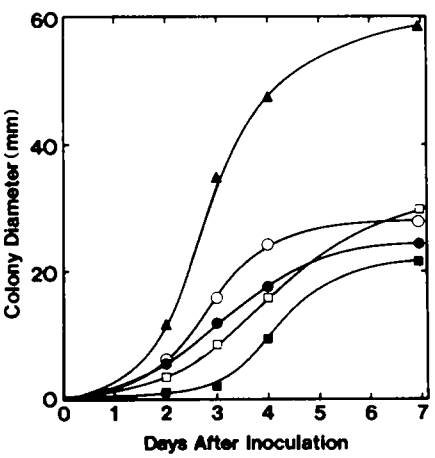


Fig. 2. Growth of *M. mucedo* (▲), *B. cinerea* (○), *A. alternata* (●), *C. coccodes* (□) and *M. fructicola* (■) colonies on water agar containing 5 mM glucose. Each value represents the mean of 16 colonies.

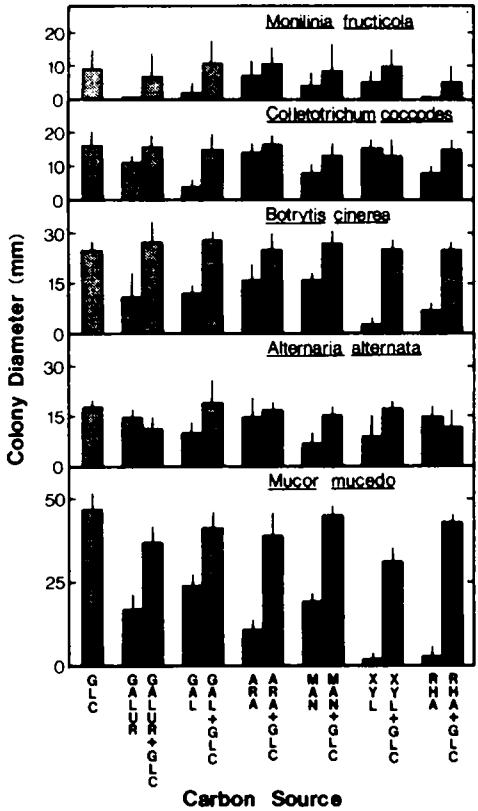


Fig. 3. Growth of fungal colonies after incubation for 4 days on water agar containing various monosaccharides (5 mM). Sugar abbreviations are as follows: glc, glucose; galur, galacturonic acid; gal, galactose; ara, arabinose; man, mannose; xyl, xylose; rha, rhamnose. Vertical lines at the top of bars represent the S.D. Each value represents the mean of 16 colonies.

the wall-related monosaccharides without glucose. It is interesting that galacturonic acid and rhamnose, major components of the pectic polysaccharides of plant cell walls, were unable to support any growth of *M. fructicola* cultures. Rhamnose and xylose were particularly poor carbon sources for the growth of *M. mucedo* and *B. cinerea* cultures.

The results of this study show that, under these conditions, substantial variation exists in the ability of fungal pathogens to utilize plant cell wall-related monosaccharides for growth. Because many wall-degrading enzymes produced by fungi during invasion cleave single residues from polysaccharides, the monosaccharide constituents of wall polymers may be available for metabolism by fungi [3,11]. It is possible that these monosaccharides affect the growth and development of fungi by means other than through their metabolism as carbon sources i.e., as effectors [3,11,12]. Clearly, further studies are necessary to explore the interaction between pathogenic

fungi and the metabolism of host cell wall sugars. Studies of the growth of fungal pathogens on native plant cell wall fractions should be particularly useful.

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